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***RESEARCH ARTICLE*.....**

Toxico-pathological studies on experimentally induced lead acetate toxicity in broiler chickens with protective effect of *Opuntia elatior* and *Withania somnifera*

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ABSTRACT..... The present study was carried out to evaluate the toxico-pathological manifestation of lead acetate toxicity in broiler chickens with protective effect of *Opuntia elatior* fruit juice and extract of *Withania somnifera*. Lead acetate was administered at dose rate of 500 ppm in feed, P.O. for 21 days while *Opuntia elatior* fruit juice (3 ml/kg, P.O) and hydro-alcoholic extract of *Withania somnifera* (100 mg/kg) were administrated alone and in combination for consecutive 21 days in chickens of treatment groups. Symptoms of toxicity were observed after few days in chickens received only lead acetate. There was non-significant decrease in feed intake and body weight gain in birds of lead acetate treated group. Upon gross pathological examination, liver of birds from lead acetatetreatment group shown paleness, enlargement and fatty changes; whereas, kidneys shown haemorrhagic lesions and atrophy. Upon microscopic examination, liver of birds treated with lead acetate only showed varying degree of degenerative changes as well as vascular changes. Intestine of birds from lead treatment group showed denaturation and necrosis of tips of intestinal villi. No appreciable histopathological lesions were observed in the spleen and heart of birds in all treatment groups. In conclusion, administration of *Opuntia elatior* and *Withania somnifera* for longer period may improve performance of broilers chickens as well as act as protectant to organs.

KEY WORDS..... *Opuntia elatior, Withania somnifera*, Toxico-pathology, Lead acetate, Chickens

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INTRODUCTION.....

Poultry sector has been growing with increase in broiler meat production and table egg consumption. Every poultry farm has its particular risk profile for the introduction of pathogens, development of disease, and spread of disease which has been influenced by a number

of factors like types of micro-organisms, nutritional imbalances and exposure to environmental pollutants. Such conditions may cause ailments with hepatotoxicity, nephrotoxicity and other complications.On other side, complementary and alternative medicine has made significant inroads as an accessory modality in toxicosis, providing a feasible option for toxicosis control, increasing the body's ability to fight against the disease, relieve side effects caused by drugs, prevention of recurrence, boosting the immune system, reducing stress and improving quality of life and general well-being (Kantor, 2009). Many herbal plants including *Opuntia ficus indica* and *Withania somnifera* were found to have pharmacological property in curing symptoms of toxicity to major organs (Kirtikar and Basu, 1999; Galati *et al*., 2003; Dok-Go *et al*., 2003 and Kuti, 2004).

Lead as an environmental pollutant can be a better candidate to produce model for hepatotoxicity and nephrotoxicity in birds (Patrick, 2006 and Alghazal *et al*., 2008). Looking to the pharmacological properties of different varieties of *Opuntia* spp. and *Withania somnifera*, the present study was planned to evaluate the protective effect of *Opuntia elatior* and *Withania somnifera* against lead acetate induced toxico-pathological effects in broiler chickens.

RESEARCH METHODS.....

Experimental birds :

Forty colour broiler chicks were procured from Intensive Poultry Development Block (IPDB), Government of Gujarat, Makarba, Ahmedabad, Gujarat, India and kept under observation before starting experiment at the age of 3 weeks. Vaccine against Newcastle disease (R2B – Mukteshwar Strain - Live Virus) was given at 5th day of age by intraocular route and vaccine against infectious bursal disease (Standard Type 1, Intermediate invasive Strain - Live Virus) was given in drinking water at 14th day of age as per standard method described by manufacturer. Both vaccines of Hester Biosciences Limited, Ahmedabad, Gujarat, India were used. The experimental protocol (No. JAU/JVC/IAEC/SA/01/ 2015) was approved by the Institutional Animal Ethics Committee (IAEC).

Juice and extract preparation :

Opuntia elatior fruits were collected from local market of Junagadh, Gujarat, India. Fruits were cleaned properly and seeds were removed and Juice was made and stored at -20° C for further use in experiment. Powder of roots of *Withania somnifera* was obtained from Ayurvedic pharmacy nearby Gujarat Ayurved University, Jamnagar. The powder was used to prepare hydro-alcoholic extract using double distilled water and methanol (40:60) and then filtered through a Whatman filter paper No.1. The filtrate was reduced to dryness in rotary evaporator under reduced pressure (Singh *et al*., 2009). Extract obtained was taken in petri plate and then kept in desiccator for drying. The extract was than stored at -20 °C till further use in experiment.

Experimental design :

Forty broiler chickens were randomized based on body weight in five groups (C_1, C_2, T_1, T_2 and T_3). Birds of group C_1 were kept as control. Birds of group C_2, T_1, T_2 and T_3 were given feed mixed with lead acetate at level of 500 ppm for 21 days of experiment. Birds of group T_1 and T_2 were administrated with *Opuntia elatior* fruit juice at dose rate of 3 ml/kg body weight, P.O. and hydro-alcoholic extract of *Withania somnifera* at dose rate of100 mg/kg body weight, P.O., respectively. Birds of group T_3 were administrated with *Opuntia elatior* fruit juice at dose rate of 3 ml/kg body weight, P.O. along with hydro-alcoholic extract of *Withania somnifera* at dose rate of100 mg/kg body weight, P.O. All birds during the treatment period were examined daily for abnormal physical and behavioral changes as well as mortality (if any) due to lead toxicity.

Body weight and feed consumption :

The body weight of individual bird was recorded daily to assess the body weight gain during the experimental period. Feed consumption by experimental birds and body weight-feed consumption ratio were also calculated.

Gross pathological examination and collection of tissue samples :

All birds were humanly sacrificed and dressed on 22ndday of study in the confined disinfected pathology laboratory and were subjected to gross pathological examination. Pathological changes were observed by systemic approach (*i.e.* gross changes in organ size, shape and any visible pathological lesions). For

histopathological examinations, tissues from liver, spleen, kidney, heart, lung, small and large intestine were collected in 10 per cent formalin.

Histopathology :

The formalin fixed tissues were subjected to paraffin wax embedding for tissue sectioning. Sections of each tissue collected were cut at approx. 6-8 microns thickness with automatic section cutting machine semi-automated rotary microtome (Leica Biosystems, Germany) and were stained with hematoxylin and eosin (H and E) stain (Luna, 1968). The H and E stained slides were observed under microscope (10 and 40 x) and microscopic pathological lesions were recorded.

Statistical analysis :

The numeric data obtained were presented as means ± standard error (SE). Data were analyzed statistically by one way ANOVA and different treatment group means were compared by Duncan's multiple range tests to observe difference among the treatments.

RESEARCH FINDINGS AND ANALYSIS.....

No apparent clinical signs of toxicity were observed in birds of any treatment group upto 10 days. After 10 days, clinical symptoms (mild depression, reduced feed

Table 1: Average feed consumption (g/day/bird) of experimental birds of different groups

Group	1st week (Mean ± SE)	2nd week (Mean ± SE)	3rd week (Mean ± SE)
C_1	115.89 ± 1.12[a]	122.79 ± 2.96 [a]	137.36 ± 1.41 [a]
C_2	100.32 ± 1.12[a]	99.34 ± 1.45 [b]	98.20 ± 1.91 [b]
T_1	117.80 ± 0.7 [a]	124.29 ± 3.14 [a]	138.41 ± 1.67 [a]
T_2	110.84 ± 1.15[a]	113.52 ± 1.36 [a]	123.00 ± 1.37 [a]
T_3	113.63 ± 0.52 [a]	122.77 ± 2.12 [a]	135.70 ± 1.73 [a]

Table 2: Average body weight gain (g/day/bird) in experimental birds of different groups

Group	1st week (Mean ± SE)	2nd week (Mean ± SE)	3rd week (Mean ± SE)
C_1	53.15 ± 3.20[a]	47.37 ± 6.42[a]	88.41 ± 1.70[a]
C_2	43.43 ± 3.28[a]	33.39 ± 3.19[a]	80.25 ± 3.09[a]
T_1	52.68 ± 2.30[a]	44.69 ± 3.13[a]	96.96 ± 2.12[a]
T_2	47.61 ± 2.48[a]	33.01 ± 3.16[a]	88.88 ± 2.18[a]
T_3	46.34 ± 2.88[a]	38.52 ± 2.82[a]	95.41 ± 3.39[a]

Table 3: Feed conversion ratio of experimental birds of different groups

Group	1st week (Mean ± SE)	2nd week (Mean ± SE)	3rd week (Mean ± SE)
C_1	2.18 ± 0.35[a]	2.59 ± 0.46[a]	1.55 ± 0.83[a]
C_2	2.31 ± 0.27[a]	2.97 ± 0.45[a]	1.22 ± 0.62[a]
T_1	2.24 ± 0.34[a]	2.78 ± 1.00[a]	1.43 ± 0.79[a]
T_2	2.33 ± 0.47[a]	3.44 ± 0.43[a]	1.38 ± 0.63[a]
T_3	2.45 ± 0.18[a]	3.19 ± 0.75[a]	1.42 ± 0.51[a]

Table 4: Effect of daily oral administration of *Opuntiaelatior* juice (3 ml/kg/day) and *Withaniasomnifera* extract (100 mg/kg/day) on organweight of chickens treated with lead acetate (500 ppm in feed) for 21 days

Organ weight (g)	Treatment groups				
	C_1	C_2	T_1	T_2	T_3
Liver	40.04 ± 0.78[a]	39.78 ± 1.08[a]	33.95 ± 4.46[a]	36.59 ± 0.44[a]	34.16 ± 0.72[a]
Kidney	12.46 ± 0.36[a]	11.11 ± 0.26[a]	11.67 ± 0.22[a]	11.51 ± 0.18[a]	11.31 ± 0.13[a]
Heart	6.70 ± 0.46[a]	6.32 ± 0.45[a]	6.79 ± 0.21[a]	6.97 ± 0.15[a]	6.72 ± 0.18[a]
Spleen	3.83 ± 0.29[a]	3.66 ± 0.24[a]	4.07 ± 0.08[a]	4.06 ± 0.11[a]	3.77 ± 0.14[a]
Lung	10.73 ± 0.23[a]	11.12 ± 0.24[a]	11.53 ± 0.27[a]	11.06 ± 0.10[a]	11.41 ± 0.23[a]

intake, dullness and rough feathers) were observed in birds who received only lead acetate. Besides this no mortality was observed in any experimental group.These findings were in agreement with previous report of Vengris and Mare (1974). During the experiment, non-significant decrease in feed intake and body weight gain in birds of lead acetate treatment group was observed (Table 1and 2). No significant (P>0.05) alterations in feed conversion ratio of birds of treatment groups were observed compared to those of control group (Table 3). This little alteration in feed consumption was due to effects on metabolic activity. Sipmson *et al*. (1970) also observed similar findings. Although *Opuntia elatior* and *Withania somnifera* along with lead acetate treated birds did not show any alteration in feed consumption. The results of present study are in agreement with previous report by Costa *et al*. (2012). Weights of liver, kidney, heart, lung and spleen of birds of any treatment group were found with non-significant difference (Table 4).

Upon gross examination, liver of chickens treated with lead acetate only shown paleness, enlargement and fatty changes (Fig. 1). While birds of group T_1, T_2 and T_3 shown slight enlarged and congested liver. Whereas, macroscopic view of kidneys shown haemorrhage and atrophy (Fig. 2). Kidneys of birds belong to group T_2 and T_3 shown slight congestion. Gross examination of spleen, heart, intestine and lung revealed no appreciable lesions in chickens of all groups. These findings are in accordance with previous work of suradkar *et al.* (2009).

Upon microscopic observation, liver of chickens

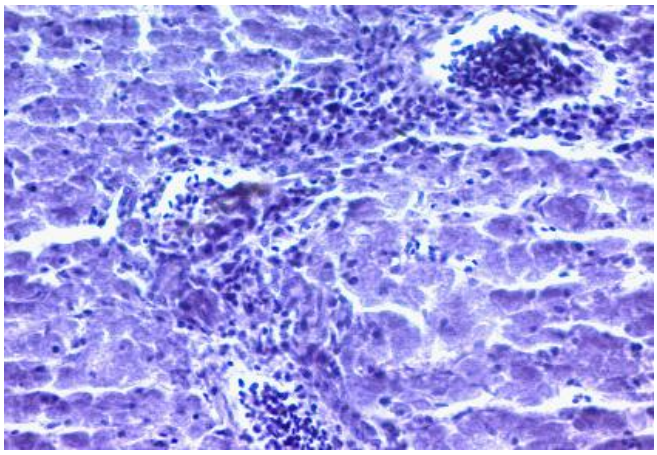

Fig. 1 : Microscopic view of liver: infiltration of inflammatory cells alongwith necrosis of hepatocytes and loss of normal architecture of parenchyma in group C_2 (H and E x 400)

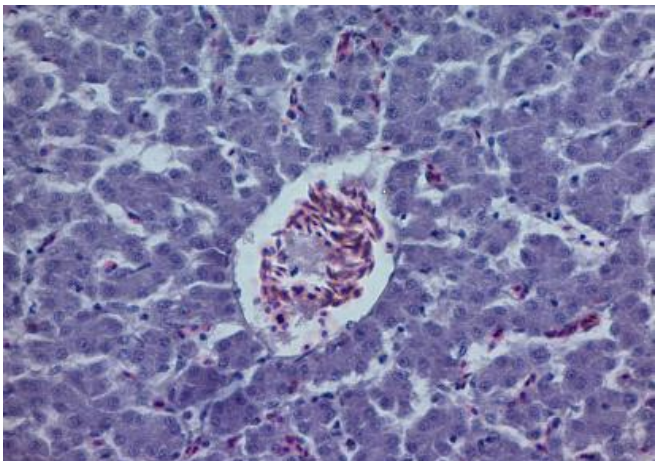

Fig. 3 : Microscopic view of liver showing moderate congestion in the central vein in Group T_3 compared to group T_2 (H and E x 400)

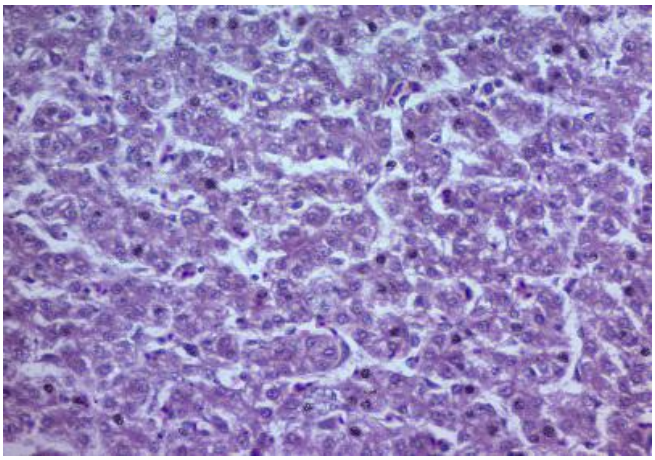

Fig. 2: Microscopic view of liver showing the vacuolar degeneration with loss of normal architecture in group T_1 (H and E x 400)

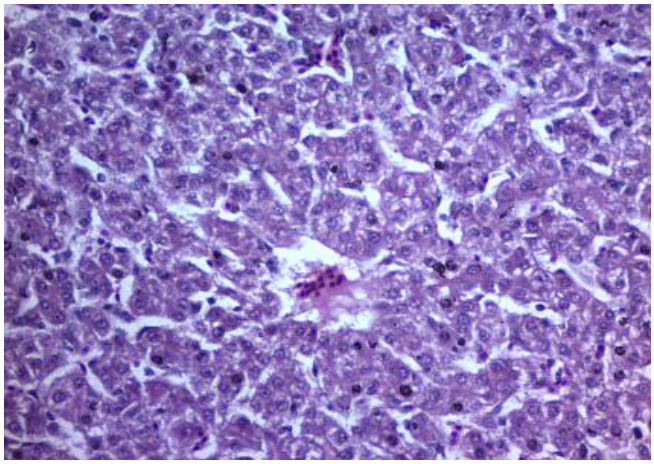

Fig. 4 : Microscopic view of liver showing slight vacuolar degeneration and congestion in group T_3 (H and E x 400)

treated with lead acetate only (group C_2) showed varying degree of degenerative changes as well as vascular changes like infiltration of inflammatory cells with necrosis of hepatocytes, loss of normal architecture of parenchyma (Fig. 1). Vacuolar degeneration with loss of normal architecture was observed in liver of chickens of group T_1 (Fig. 2). Whereas only moderate congestion in the central vein in liver with slight vacuolar degeneration were observed in liver of birds of group T_2 and T_3 (Fig. 3 and 4). Appearance of inflammatory cells in the hepatic tissue might be due to the interaction proteins and enzymes of the hepatic interstitial tissue which interfering with the antioxidant defense mechanism and leading to reactive oxygen species (ROS) generation which in turn may imitate an inflammatory response. However, microscopic view of liver of chickens received *Opuntia elatior* fruit juice and *Withania somnifera* along with lead acetate shown moderate congestion in the central vein as well as vacuolar degeneration. Similar ameliorating effects have been observed by Galati *et al.* (2005). Hepatoprotective effect of the *Opuntia elatior* fruit juice might be due to the presence of ascorbic acid, betalains, betacyanins, rutin and isorhamnetin derivatives.

Microscopic view of kidneys of group C_2 showed heavy infiltration of polymorphs and mononuclear cell in between tubules (Fig. 5). While microscopic view of kidneys belongs to group T_1 showed hypercellurity of glomeruli and mild congestion (Fig. 6). Hypercellurity of glomeruli and degenerated tubular epithelium were observed in kidney of chickens of group T_2 (Fig. 7). While,

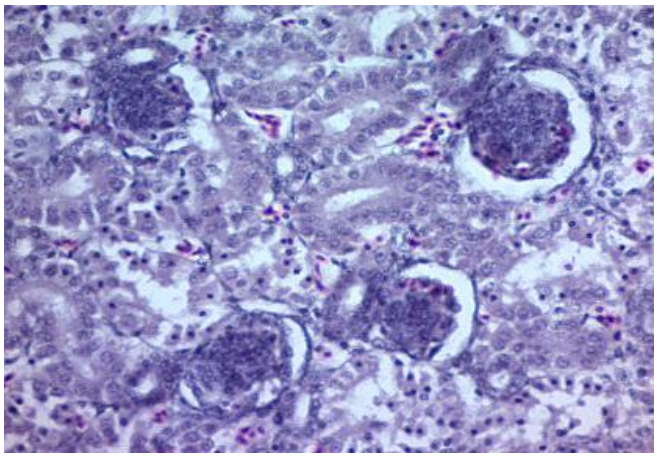

Fig. 5 : Microscopic view of kidney showing increased the space in glomuruli along with degeneration and necrosis of tubular epithelium in group C_2 (H and E x 400)

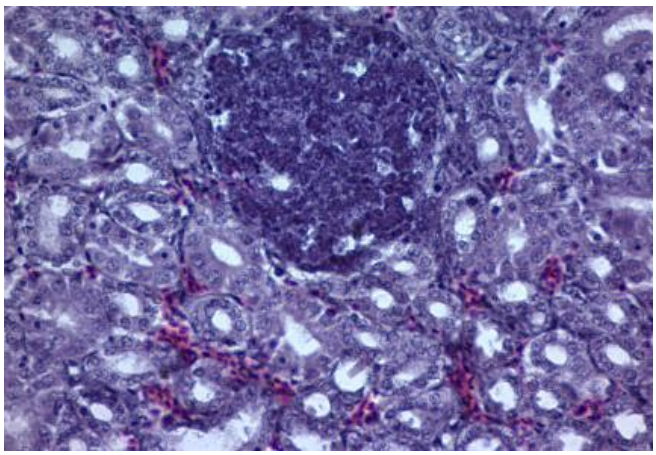

Fig. 6 : Microscopic view of kidney showing hypercellurity of glomeruli and mild congestion in group T_1 (H and E x 400)

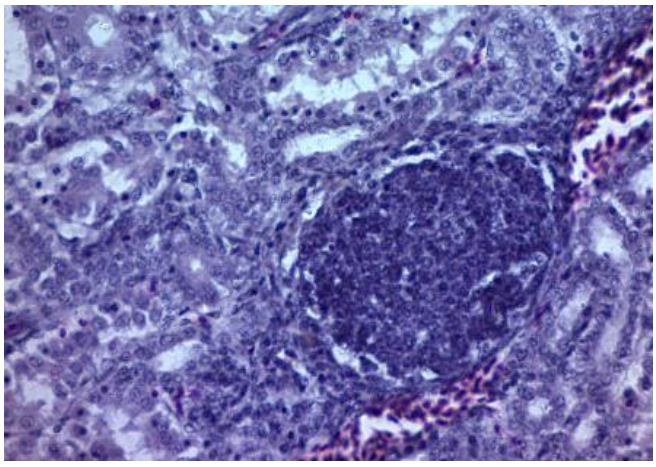

Fig. 7 : Microscopic view of kidney showing hypercellurity of glomeruli and degenerated tubular epithelium in group T_2 (H and E x 400)

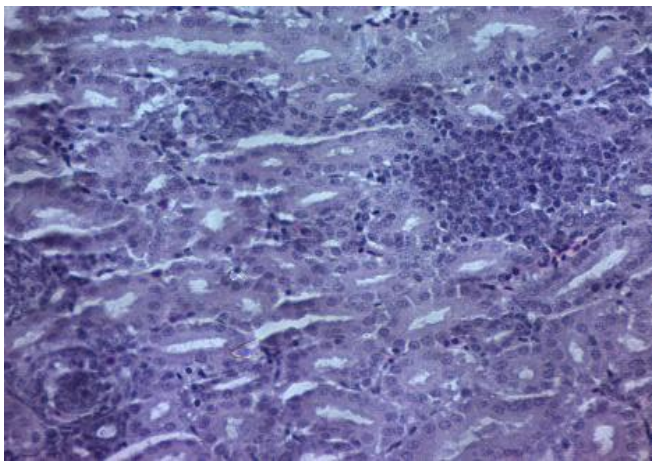

Fig. 8 : Microscopic view of kidney showing mild infiltration of polymorphs and mononuclear cell in between tubules in group T_3 (H and E x 400)

kidneys of group T_3 showed moderate congestion and mild degeneration of tubular epithelium (Fig. 8). These findings are in agreement with previous study of Sujatha *et al.* (2011). These histopahological lesions found in kidneys might be due to accumulation of lead-protein complex which causes discernible changes in proximal tubular linings of cells (Goyer, 1988). Lead deposited predominantly in the proximal tubule may also be considered the main reason for its deleterious effects on the cortex of the kidney. However, in our study, microscopic view of kidneys of *Opuntia elatior* fruit juice and *Withania somnifera* along with lead acetate treated birds shown moderate congestion and mild degeneration of tubular epithelium. These findings are in close agreement with previous report from Alimi *et al*. (2013).

Intestine of birds from group C_2 (lead treated) showed denaturation and necrosis of tips of intestinal villi. While intestine of birds from group T_1 revealed infiltration of polymorphs and mononuclear cells in the lamina propria. Whereas, in group T_2 and T_3, mild infiltration of polymorphs and mononuclear cells in the lamina proparia and proliferative villi in intestine were observed. No appreciable histopathological lesions were observed in the spleen and heart of birds of all treatment groups.

In conclusion,daily administration of *Opuntia elatior* fruit juice and *Withania somnifera* extract in chickens may be beneficial for protection against damaged caused by toxicant in liver and kidney. However, molecular marker assisted controlled study in large population is needed for further validation of ameliorative effects during toxicosis in chickens.

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